

Quantitative estimation of photosynthetic pigments using new spectral indices

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Abstract: Foliar chlorophylls are the most important pigments related to the physiological function of plants. Quantitative estimation of photosynthetic pigments can provide important information about relationships between plants and their environmental conditions. In this study, new spectral indices were designed to enhance spectral resistance to noise, using the area of the spectral curve and axis. The specific area was around the red edge (R_{daa}), instead of the sum of the first derivative of the spectrum, specifically the area of red edge (R_{da}). Meanwhile, three reference indices were also introduced as non-sensitive bands of chlorophylls. The results show that by dividing spectral references, a kind of re-projection, the spectral indices can be calibrated to allow direct and reasonable comparisons of the results. The sensitivity of these reference indices to chlorophylls was also evaluated in this study. The regression results show that R_{daa} and its derivatives are highly related to chlorophylls and resistant to noise.

Key words: chlorophyll; spectral indices; red edge; indicator; paddy rice

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Introduction

Photosynthesis, the process of converting light energy to stored chemical energy that powers the biosphere, is one of the most important biochemical processes for plants. The chlorophylls have an integral relationship with the physiological function of leaves and consequently are the most important pigments (Richardson et al. 2002; Gitelson et al. 2003). From a physiological perspective, chlorophyll content is related to the amount of solar radiation and total leaf nitrogen, and provides valuable information about the physiological status of plants (Sims and Gamon 2002; Blackburn and Ferwerda 2008). From an applied perspective, low concentrations of chlorophyll directly limit photosynthetic potential and hence primary production (Richardson et al. 2002; Zou et al. 2010). Therefore, quantifying the chlorophyll content can provide important information about the relationships between plants and their environmental conditions (Spitale 2009). Although there are well established methods for chlorophyll content determination through destructive sampling, this method is relatively fast and avoids damage to the growing leaves.

Quantitative estimation of chlorophyll content with spectral indices is promising because pigments, such as chlorophyll, have different spectral absorption features (Bannari et al. 2007; Boyd and Foody 2011). Many spectral indices or models exist, such as the Chlorophyll Absorption Ratio Index (CARI) (Broge and Mortensen 2002a), Triangular Vegetation Index (TVI) (Broge and Leblanc 2000b), Photochemical Reflectance Index (PRI) (Gamon et al. 1997; Barton and North 2001) and Carotenoid Reflectance Index (CRI) (Gitelson et al. 2002). Specifically, red edge wavebands were utilized to indicate chlorophyll content. Chappelle et al. (1992) estimate chlorophyll content using 675 nm and 700 nm bands (Chappelle et al. 1992). Gitelson et al. (2006) found the ratio of reflectance at 750 nm to 700 nm (R_{750}/R_{700}) was directly proportional to chlorophyll concentration (Gitelson, Merzlyak 1996). Datt (1998) concluded that 550 nm, 672 nm and 708 nm were the best bands for estimating

chlorophyll a, chlorophyll a + b, and total carotenoids (Datt 1998).

Based on these reported wavebands, many spectral indices have been developed (Gitelson et al. 2002; Blackburn 1999; Daughtry et al. 2000; Gamon et al. 1992; Gitelson et al. 1996). However, these spectral indicators are individual-band-based (i.e., they use only narrow spectral bands), and thus are sensitive to noise (Bannari et al. 2007).

By applying the average of reflectance from 520 nm to 550 nm and 695 nm to 705 nm, Gitelson et al. (2002) developed a reciprocal reflectance model, which was closely related to the total chlorophyll content in a leaf (Gitelson et al. 2002). Another index used by Gitelson et al. (2006) was the average of reflectance from 695–740 nm and from 750–800 nm. The general form of this model was given as $\ln[R(\lambda)^{-1} - (R_{NIR})^{-1}]$ (R was the spectral reflectance at band λ or NIR). While this model was designed to reduce noise, essentially it is still based on narrow spectral bands, such that when noise was suppressed, useful information was lost, resulting in a reduction in the sensitivity of the index. In addition, most spectral indices were developed based on remote-sensing images or tree-canopy level data that are seriously impacted by external factors. For example, water vapor and aerosol absorption are not present with ground-based fine-resolution data collected at leaf level with equipment that has a low signal-to-noise ratio (e.g., Analytical Spectral Devices FieldSpec Pro FR spectrometer). To provide sufficient sensitivity for detecting small variations in chlorophyll, a broad spectra range is required (Gitelson et al. 2002). In present study we developed new spectral indices as indicators of chlorophylls.

Materials and methods

Data collection

A total of 79 leaf samples of paddy rice in the tillering stage were collected. The spectral reflectance data of the rice were obtained using an ASD spectrometer with the contact probe and leaf-clip, in order to reduce the effects of atmosphere on leaf-level spectra. The center of the field of view (FOV) of the fiber cable was located at the center of the middle front of the second leaf counted from the core of the rice plant. The principal vein of the leaf was vertical to the view line from the fiber cable. Two measurements were taken on one leaf along the principal vein. To reduce the heat damage to the leaf, every measurement was recorded as the average of 5 consecutively acquired spectra. All the leaf samples were stored in a box with ice and transported to the laboratory within 2 hours for analysis of the chlorophyll a and b concentrations.

Pigment absorption

Fig. 1 shows the absorption positions of the photosynthetic pigments. Chlorophyll pigments have a strong absorption within the visible spectrum. There is a reflected peak at the green bands (centered at 550 nm), while at red bands (centered at 670 nm)

there is very strong spectral absorption where the absorption rate is nearly 95%. At the red edge band (centered at 700 nm), the reflection rate increases rapidly with an increase in wavelength. Chlorophyll a, has two reflection peaks: one is located at 400–500 nm and the other is at 650–700 nm. The former has a stronger reflection rate than the latter with an increase in 400–500 nm. However, this reflection peak overlaps with the reflection peak of chlorophyll b and carotenoids. Consequently, estimates of chlorophyll b and carotenoids cannot be as accurate as those of chlorophyll a. Therefore, empirical models for estimation of chlorophyll generally choose bands around 500 nm and 700 nm (Sims and Gamon 2002).

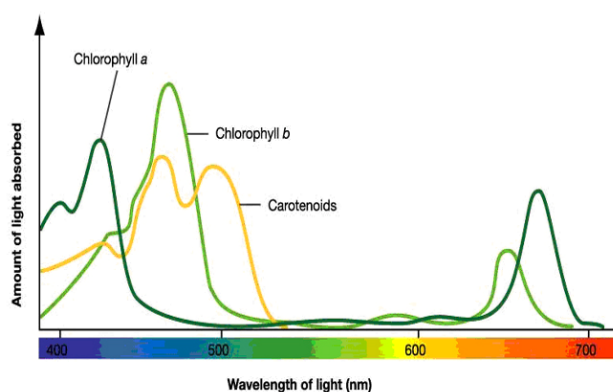


Fig. 1 Typical photosynthetically active radiation (PAR) action spectrum, shown beside absorption spectra for chlorophylls a, b, and carotenoids.

Design of new spectral indices as indicators of chlorophylls

The red edge is a shift of vegetation reflectance from red bands to near infrared bands. A change in chlorophyll concentration may result in a red shift (Curran et al. 1995; Blackburn and Milton 1995). High concentrations of chlorophyll increase absorption in the red region and push the red edge to longer wavelengths. This is a widely accepted spectral feature of vegetation, which is highly related to chlorophyll content in leaves. The red edge generally ranges from 670 nm to 780 nm (Clevers 2004), but when chlorophyll concentration decreases, the red edge moves to shorter wavelengths and the range of the red edge increases dramatically (Fig. 2). This shift can reduce noise and is sensitive to changes in chlorophyll. Thus, the wavelengths within the red edge range provide be an ideal parameter upon to build a chlorophyll prediction model.

The red-edge position (REP) is generally defined as the point with the largest slope (the inflection point) of the spectral reflectance curve between the red and NIR (near infrared) wavelengths (Clevers 2004; Dawson and Curran 1998). In specific, this refers to the band (position) where the maximum of the first derivative of the original curve located. The REP can be studied by plotting $dR/d\lambda$ (R was the spectral reflectance at band λ), as a function of λ . Equation 1 defines REP with 1 λ -step. Each step in the derivative equation controls effects of data smoothing. $R(\lambda_i)$ is the spectral reflectance at band $R(\lambda_i)$. The larger it is, the smoother the derivative curve is.

$$\frac{dR(\lambda_i)}{d\lambda} = \frac{\left(\sum_{j=i-1}^i R(\lambda_j) - \sum_{j=i}^{i+1} R(\lambda_j) \right)}{2\Delta\lambda} \quad (1)$$

Previous research work defined the area of the red edge (R_{da}) as the sum of the first derivative of the spectrum within the red-edge range (equation 2) and found R_{da} to be highly related to chlorophyll (Filella and Penuelas 1994).

$$R_{da} = \sum_{680-780nm} S_{um}(dR/d\lambda) \quad (2)$$

Where, R is the spectral reflectance at band λ . According to the definition proposed by Filella et al. (1994), R_{da} is the sum of the gradients. In this study, the area around the red edge (R_{daa}) is redefined as the sum of reflectance in the red edge range as in Equation 3.

$$R_{daa} = \sum_{680-780nm} S_{um}(R) \quad (3)$$

Plant leaves have a unique characteristic in the red and near infrared red spectral wavelength. Photosynthetic pigments strongly absorb wavelengths in the red range and are unable to absorb wavelengths in the near infrared range. Previous researchers have shown that the regression of foliar contents and spectral parameters can be calibrated and noise can be suppressed by using non-sensitive bands as reference bands. The ratio of reflectance at 550 nm (R_{550}) and 700 nm (R_{700}) is constant at different level of chlorophyll concentrations. Hoel and Solhaug (1998) pointed out that chlorophyll does not absorb wavelengths of 940 nm (Hoel and Solhaug 1998). The chlorophyll meter SPAD 502 (Minolta Camera Co. Ltd. Japan) was designed based on this idea. Furthermore, Sims and Gamon (2002) found the spectral reflectance at 445 nm (R_{445}) was constant until total chlorophyll concentration dropped below 4% and R_{445} would be another good reference band (Sims and Gamon 2002). Thus, the three band regions that center at 445 nm, 700 nm and 940 nm were chosen for consistent absorbance regardless of chlorophyll concentration. In order to match R_{daa} and resistance to noise, the reference indices were extended from a single band to a range of bands. Therefore, new indices for chlorophyll prediction (I_{nd}) defined by the spectral area index are as follows:

$$I_{nd1} = R_{daa} / (R_{440-450}) \quad (4)$$

$$I_{nd2} = R_{daa} / (R_{540-560}/R_{690-710}) \quad (5)$$

$$I_{nd3} = R_{daa} / (R_{930-950}) \quad (6)$$

$R_{440-450}$, $R_{540-560}$, $R_{690-710}$, and $R_{930-950}$ are the sum of spectral reflectance at 440–450 nm, 540–560 nm, 690–710 nm, and 930–950 nm, respectively. By comparison, R_{daa} can be replaced by R_{da} in calculations of I_{nda1} , I_{nda2} and I_{nda3} . When analyzing the

red-edge shift in Fig. 2, it can be seen that the amplitude would be not very large. Generally, the amplitude of the red-shift ranges from several nanometers to tens of nanometers; therefore, when calculating the area of the red edge, including the whole reflectance data with edges, the sensitivity of the area responding to changes in chlorophyll would be reduced largely 1. In order to overcome this short fall, revised area indices were proposed in this section based on the index of I_{nd} . Generally, the red-edge position is located in the range of 700–730 nm. The spectral responses to chlorophyll changes in this range, if any, are sharp, and are the main responding spectra to chlorophyll content. Therefore, by adjusting the scope of the edge for calculating the area, the sensitivity of the area index responding to chlorophyll could be increased. Thus, the spectral area indices of I_{nd} were redefined as follows:

$$I_{nd1} = R_{daa}' / (R_{440-450}) \quad (7)$$

$$I_{nd2} = R_{daa}' / (R_{540-560}/R_{690-710}) \quad (8)$$

$$I_{nd3} = R_{daa}' / (R_{930-950}) \quad (9)$$

$$R_{daa}' = \sum_{700-730nm} S_{um}(R) \quad (10)$$

The abbreviations related to these indices are as follows: The indices are defined in the red edge [680, 780 nm]: R_{da-o} is the sum of first derivate of spectral reflectance in the red edge; I_{nda1-o} , I_{nda2-o} and I_{nda3-o} are based on R_{da-o} . R_{daa-o} represents the sum of spectral reflectance in the red edge. The indices defined in the range of [700, 730 nm]: R_{da} is the sum of first derivate of spectral reflectance in [700, 730nm], R_{daa} is the sum of spectral reflectance in [700, 730nm], and I_{nd1} , I_{nd2} and I_{nd3} were based on R_{daa} .

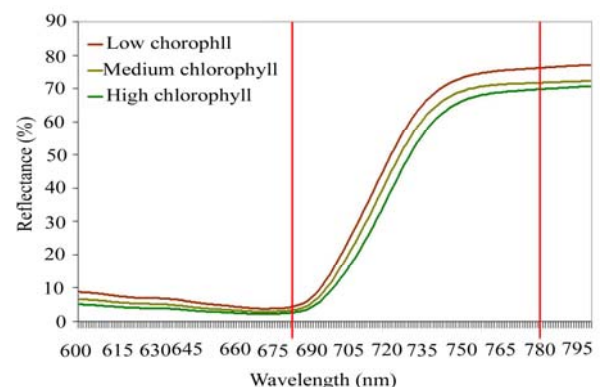


Fig. 2 Red edge area (marked by red line).

Validation of new spectral indices

A linear regression model was used to describe the relationship between new spectral indices and chlorophyll content. Five statistical parameters were selected to compare the relationships. The r^2 represents the proportion of variance in the dependent variable explained by the regression model. “Standard error of the estimate” shows the predicted error caused by the model. In

addition, the results of the estimated residual were also assessed and the “standard deviation” of the residuals was given. The “Durbin-Watson test” was used to test whether the residuals are auto-correlated. It provides a value ranging from 0 to 4. A value near 2 indicates non-autocorrelation, a value toward 0 indicates positive autocorrelation, and a value towards 4 indicates negative autocorrelation. If the residuals are auto-correlated, it implies that the linear regression equation does not significantly explain the relationship between the variables. The performance of new spectral indices was also compared with the spectral index of Datt (1998), which had proven to be superior to other existing spectral indices when used as an indicator of chlorophyll content (Li 2011).

New spectral indices were designed based on the idea that the area was able to reduce and suppress noise better than the indices based on narrow bands (i.e., individual bands). For further evaluation, the noise was added to the original spectral reflectance. Random noises were generated by the function of “randn” under Matlab software and added to the spectral reflectance data. They were fitted to a normal distribution of which mean and standard deviation was 0 and 1, respectively.

Results and discussion

Sensitivity analysis of existing chlorophyll reference indices

Table 1 shows the relationship between the chlorophyll content and the reference indices. The three existing indices that were tested showed no relationship to chlorophyll, and were insensitive to chlorophyll.

Table 1. Sensitivity analysis for reference indices to chlorophyll content

Index	Regression model	r^2
Spectral area ($R_{435-455}$)	$Y_{chl\ a}=3.888-1.362x$	0.194
	$Y_{chl\ b}=1.193-0.398x$	0.176
	$Y_{chl\ a+b}=5.081-1.760x$	0.194
$R_{540-560}/R_{690-710}$	$Y_{chl\ a}=1.341+1.008x$	0.008
	$Y_{chl\ b}=0.710+0.043x$	0.000
	$Y_{chl\ a+b}=5.256-0.290x$	0.005
Spectral area ($R_{930-950}$)	$Y_{chl\ a}=0.079+0.043x$	0.079
	$Y_{chl\ b}=1.778-0.104x$	0.107
	$Y_{chl\ a+b}=7.034-0.393x$	0.088

Relationship between new indices and chlorophyll

Table 2, 3 and 4 show the results of correlation analysis between the new tested indices and chlorophyll. I_{nd2} , I_{nd3} , I_{nd3-o} , R_{daa} and R_{daa-o} correlated significantly with the content of chlorophyll a, chlorophyll b and chlorophyll a+b. For prediction of chlorophyll a, the best indicator was I_{nd2} , the correlation coefficient as high as -0.81. The result of I_{nd3} and R_{daa} were similar, because the reference of I_{nd3} was concentrated around 1, thus I_{nd3} was approximately equal to R_{daa} . I_{nd3} and R_{daa} got the same results when they were used to estimate chlorophyll b and chlorophyll a+b.

Applying the redefined area as an indicator of chlorophyll was more efficient, more sensitive and more stronger related to chlorophyll than using the original one.

Table 2. Regression results between spectral indices and content of chl a $Y=\text{content (chl a) mg}\cdot\text{g}^{-1}$ (per fresh weight)

Index (x)	Pearson correlation	Regression Model			Residual	
		Fit equation (Unstandardized)	r^2	Std. error of the estimate	Std. deviation	Durbin-Watson test
I_{nd1-o}	0.160	##	##	##	##	##
I_{nd1}	-0.168	##	##	##	##	##
I_{nd2-o}	-0.368	##	##	##	##	##
I_{nd2}	-0.81	$Y=5.292-0.400x$	0.463	0.5670	0.5656	1.452
I_{nd3-o}	-0.733	$Y=12.297-0.3129x$	0.537	0.5339	0.5253	1.470
I_{nd3}	-0.728	$Y=6.48-4.956x$	0.531	0.5376	0.5288	1.325
I_{nda1-o}	0.342	##	##	##	##	##
I_{nda1}	0.361	##	##	##	##	##
I_{nda2-o}	0.080	##	##	##	##	##
I_{nda2}	0.081	##	##	##	##	##
I_{nda3-o}	0.224	##	##	##	##	##
I_{nda3}	0.269	##	##	##	##	##
R_{da-o}	-0.068	##	##	##	##	##
R_{da}	0.075	##	##	##	##	##
R_{daa-o}	-0.574	$Y=6.932-0.145x$	0.380	0.6425	0.6320	1.995
R_{daa}	-0.731	$Y=5.770-0.449x$	0.534	0.5358	0.5271	1.756
d_{att}	0.728	$Y=0.176+42.589x$	0.530	0.5380	0.5292	1.484

Notes: “##” means no or weak relationship between two variables. It is the same as other Tables

Table 3. Regression results between spectral indices and chl b content $Y=\text{content(chl b) mg}\cdot\text{g}^{-1}$ (per fresh weight)

Index (x)	Pearson correlation	Regression model			Residual	
		Fit equation (Unstandardized)	r^2	Std. error of the estimate	Std. deviation	Durbin-Watson test
I_{nd1-o}	0.130	##	##	##	##	##
I_{nd1}	-0.181	##	##	##	##	##
I_{nd2-o}	-0.331	##	##	##	##	##
I_{nd2}	-0.637	$Y=1.554-0.111x$	0.405	0.1796	0.1767	1.710
I_{nd3-o}	-0.694	$Y=3.533-0.879x$	0.481	0.1678	0.1651	1.989
I_{nd3}	-0.696	$Y=1.815-1.406x$	0.485	0.1672	0.1645	1.924
I_{nda1-o}	0.308	##	##	##	##	##
I_{nda1}	0.312	##	##	##	##	##
I_{nda2-o}	0.102	##	##	##	##	##
I_{nda2}	0.077	##	##	##	##	##
I_{nda3-o}	0.226	##	##	##	##	##
I_{nda3}	0.218	##	##	##	##	##
R_{da-o}	-0.088	##	##	##	##	##
R_{da}	0.023	##	##	##	##	##
R_{daa-o}	-0.574	$Y=2.098-0.043x$	0.329	0.1907	0.1876	1.933
R_{daa}	-0.717	$Y=1.734-0.131x$	0.514	0.1624	0.1598	1.983
d_{att}	0.738	$Y=0.084+12.814x$	0.545	0.5772	0.1546	1.935

Notes: “##” means no or weak relationship between two variables.

Table 4. Regression results between spectral indices and content of chl a+b, Y is content (chl a+b) $\text{mg}\cdot\text{g}^{-1}$ (per fresh weight)

Index (x)	Pearson correlation	Regression Model				Residual	
		Fit equation (Unstandardized)	r^2	Std. error of the estimate	Std. deviation	Std.	Durbin-Watson test
I_{nd1-o}	0.155	##	##	##	##	##	##
I_{nd1}	-0.173	##	##	##	##	##	##
I_{nd2-o}	-0.364	##	##	##	##	##	##
I_{nd2}	-0.679	$Y=8.846-0.511x$	0.461	0.7374	0.7254	1.486	
I_{nd3-o}	-0.733	$Y=15.830-4.008x$	0.538	0.6832	0.6721	1.572	
I_{nd3}	-0.730	$Y=7.963-6.362x$	0.533	0.6862	0.6750	1.437	
I_{nda1-o}	0.339	##	##	##	##	##	##
I_{nda1}	0.354	##	##	##	##	##	##
I_{nda2-o}	0.087	##	##	##	##	##	##
I_{nda2}	0.081	##	##	##	##	##	##
I_{nda3-o}	0.227	##	##	##	##	##	##
I_{nda3}	0.261	##	##	##	##	##	##
R_{da-o}	-0.074	##	##	##	##	##	##
R_{da}	0.064	##	##	##	##	##	##
R_{daa-o}	-0.582	$Y=9.030-0.189x$	0.338	0.8173	0.8040	1.973	
R_{daa}	-0.737	$Y=7.504-0.580x$	0.543	0.6792	0.6682	1.789	
d_{att}	0.740	$Y=0.261+55.404x$	0.547	0.6760	0.6650	1.551	

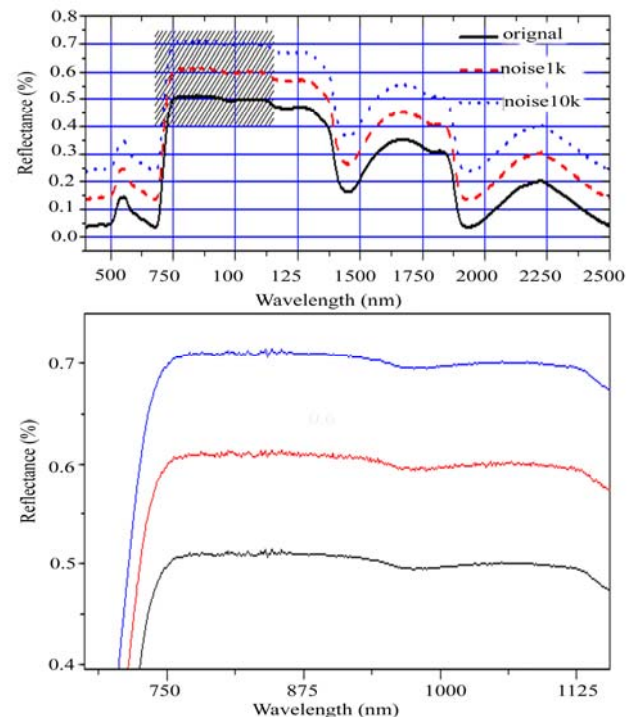
Notes: “##” means no or weak relationship between two variables.

The ratio of R_{daa} divided by the reference index was used to directly compare the results between different samples. Because of random or systematic noise picked up in the reference index, the correlation coefficient of I_{nd} and chlorophyll were smaller than that of R_{daa} and chlorophyll. Also, the stability of the reference, e.g. reasonability of its construction, would affect the result as well. However, when more than one kind of species sample is analyzed, this re-projection using the ratio of R_{daa} and the reference index is necessary. Using the ratio between R_{daa} and the reference index strengthens the relationship between I_{nd-o} and chlorophyll. For example, when estimating chlorophyll a, the coefficient of R_{daa-o} and chlorophyll a was just -0.574. When re-projecting with the reference index ($R_{930-950}$), it was enhanced to -0.733, which is much more highly correlated than the original R_{daa-o} (Table 4). This revealed that the reference index can help to reduce and suppress the influence of noise. The results showed that chlorophylls predicted by the newly defined indices were highly consistent with Datt (1998). However, the new indices contain more physical meanings and could be easily explained and understood.

Analysis of new spectral indices' sensitivity to noise

Fig. 3 shows the details of the spectral reflectance curve mixed with random normally distributed numbers. Table 5 displays the regression analysis results. Here “ R_{daa} ” and “ d_{att} ” were chosen as examples. R_{daa} is an index based on the area of the spectral curve while d_{att} is based on the reflectance of narrow bands. The correlation coefficients of R_{daa} and chlorophyll a changed only slightly with varying noise levels. For d_{att} , when noise was added,

the correlation coefficients decrease drastically. The r^2 of the regression equation of d_{att} also dropped, while the r^2 of R_{daa} remained nearly constant. These statistical results revealed that indices based on the area could help to reduce and suppress noise well.

**Fig. 3** Spectral reflectance curve mixed with different normally distributed random numbers. The black curve was the original data, the red curve was added $0.001 \times \text{random noises} + 0.1$ (for display clearly) and the blue one was add $0.0001 \times \text{random noises} + 0.2$ (for display clearly).**Table 5.** Comparison of regression results between the spectral indices based on the original signal and polluted one Y is content (chl a) $\text{mg}\cdot\text{g}^{-1}$ (per fresh weight)

Index (x)	Pearson correlation	Regression Model				Residual	
		Fit equation (Unstandardized)	r^2	Std. error of the estimate	Std. devia- tion	Std.	Durbin- Watson test
R_{daa}	-0.731	$Y=5.770-0.449x$	0.534	0.5358	0.5271	1.756	
R_{daa_10k}	-0.729	$Y=5.894-0.462x$	0.531	0.5488	0.5401	2.027	
R_{daa_1k}	-0.729	$Y=5.897-0.462x$	0.531	0.5485	0.5399	2.026	
d_{att}	0.728	$Y=0.176+42.589x$	0.530	0.5380	0.5292	1.484	
d_{att_10k}	0.660	$Y=0.364+39.763x$	0.436	0.6019	0.5925	2.020	
d_{att_1k}	0.656	$Y=0.383+39.487x$	0.431	0.6045	0.5950	2.055	

Notes: R_{daa_1k} (k means adding $0.001 \times \text{random noises}$) represents it was calculated based on the spectral reflectance data which were added $0.001 \times \text{random noises}$, while R_{daa_10k} were based on $0.01 \times \text{random noises}$. The noises were generated by the function of “randn” under Matlab software. They obey normal distribution which mean and standard deviation was 0 and 1 respectively.

Analysis of correlation between spectral indices and their sensitivity

Table 6 shows that spectral reference can help to reduce and suppress the influence from non-related variables. I_{nd3} and I_{nda3-o} were calculated using the same spectral reference but with areas. However, by re-projection the non-sensitive proportions of the spectral reflectance in R_{daa-o} were suppressed, and therefore the coefficient increased to 0.989. R_{daa-o} was less sensitive to chlorophyll. Using d_{att} as a reference, it found the correlation coefficient between R_{daa-o} and d_{att} was only 0.620 while the correlation between R_{daa-o} and R_{daa} was 0.839.

The correlation coefficient of I_{nd2} and R_{daa} was 0.947. It revealed the spectral reference of $R_{540-560}/R_{690-710}$ was more stable than the others. The correlation coefficient between d_{att} and R_{daa} and the coefficient between d_{att} and I_{nd3} were very close to each other. The correlation coefficient between R_{daa-o} and I_{nda3-o} was less than 0.6, while the correlation between R_{daa} and I_{nda3-o} was more than 0.8, namely I_{nda3-o} was closer to R_{daa} than any other index. These results indicated that it was reasonable to choose the range of [700, 730 nm] as the red-edge position location that is sensitive and highly related to chlorophyll.

Table 6. Pearson correlations analysis between new spectral indices

Item	I_{nd2}	I_{nd3}	I_{nda3-o}	R_{daa}	R_{daa-o}	d_{att}
I_{nd2}	1					
I_{nd3}	0.839(**)	1				
I_{nda3-o}	0.829(**)	0.989(**)	1			
R_{daa}	0.947(**)	0.873(**)	0.884(**)	1		
R_{daa-o}	0.826(**)	0.560(**)	0.598(**)	0.891(**)	1	
d_{att}	-0.712(**)	-0.863(**)	-0.843(**)	-0.839(**)	-0.620(**)	1

Notes: ** Correlation is significant at the 0.01 level (2-tailed).

Conclusions

In this study, 15 spectral parameters were designed to be used as chlorophyll indicators. The results showed that I_{nd1-o} and I_{nd2-o} were not related to chlorophyll. Taking into account that the red-edge position is generally located in the range of 700–720 nm, R_{daa} was revised and limited to within 700, 720 nm, and I_{nd1} , I_{nd2} , and I_{nd3} were calculated based on R_{daa} . R_{daa} proved much more sensitive and highly related to chlorophyll. I_{nd1} was not related to any chlorophyll parameters while I_{nd2} was the opposite. Because the spectral reference for I_{nd3} was approximately 1, its characteristics were similar to that of R_{daa} . The sensitivity of the spectral reference was then assessed. The results showed the R_{daa} , I_{nd2} , and I_{nd3} were related to chlorophyll. Meanwhile, the results showed that by dividing the spectral reference, a kind of re-projection or the influence from non-related variables could be reduced and suppressed. However, these new spectral indices were developed based on the spectral data of paddy rice and need to be evaluated further for application to a wide range of vegetation.

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